01-28-00

A

Practitioner's Docket No. 739-009159-US(PAR) **PATENT**

Preliminary Classification:

Proposed Class:

Subclass:

NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P. § 601, 7th ed.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of

Inventor(s): Ivo STEMMLER, Andreas BRECHT, Gunter GAUGLITZ, Michael STEINWAND

WARNING: *37 C.F.R. § 1.41(a)(1) points out:*

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

"(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title): QUANTITATIVE DETERMINATION OF ANALYTES IN A HETEROGENEOUS SYSTEM

CERTIFICATION UNDER 37 C.F.R. § 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this New Application Transmittal and the documents referred to as attached therein are being deposited with the United States Postal Service on this date January 27, 2000, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EL336861901US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Shauna Murphy

(type or print name of person mailing paper)

Shauna Murphy

Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

"WARNING: *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

1. Type of Application

This new application is for a(n)

(check one applicable item below)

☒ Original (nonprovisional)

☐ Design

☐ Plant

WARNING: *Do not use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. § 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: *Do not use this transmittal for the filing of a provisional application.*

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

☐ Divisional.

☐ Continuation.

☐ Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. §§ 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. § 112. Each prior application must also be:

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. §§ 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. §§ 120, 121 or 365(c). (35 U.S.C. § 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. §§ 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application* ***must*** *be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

☐ The new application being transmitted claims the benefit of prior U.S. application(s). Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for filing date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application

23 Pages of specification

4 Pages of claims

5 Sheets of drawing

WARNING: ***DO NOT*** *submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 C.F.R. § 1.84, see Notice of March 9, 1988 (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page . . ." 37 C.F.R. § 1.84(c)).

(complete the following, if applicable)

☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. § 1.84(b).

☐ formal

☐ informal

B. Other Papers Enclosed

_____ Pages of declaration and power of attorney

1 Pages of abstract

1 Other 1 page Drawing descriptions

4. Additional papers enclosed

☐ Amendment to claims

☐ Cancel in this applications claims _____ before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

☐ Add the claims shown on the attached amendment. (Claims added have been numbered consecutively following the highest numbered original claims.)

☒ Preliminary Amendment

☐ Information Disclosure Statement (37 C.F.R. § 1.98)

☐ Form PTO–1449 (PTO/SB/08A and 08B)

☐ Citations

☐ Declaration of Biological Deposit

☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative

☐ Special Comments

☐ Other

5. Declaration or oath (including power of attorney)

NOTE: A newly executed declaration is not required in a continuation or divisional application provided that the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47, then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. §§ 1.63(d)(1)-(3).

NOTE: A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name including family name and at least one given name, without abbreviation together with any other given name or initial, and the residence, post office address and country or citizenship of each inventor, and state whether the inventor is a sole or joint inventor. 37 C.F.R. § 1.63(a)(1)-(4).

☐ Enclosed

Executed by

(check **all** *applicable boxes)*

☐ inventor(s).

☐ legal representative of inventor(s). 37 C.F.R. §§ 1.42 or 1.43.

☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

☐ This is the petition required by 37 C.F.R. § 1.47 and the statement required by 37 C.F.R. § 1.47 is also attached. See item 13 below for fee.

☒ Not Enclosed.

NOTE: Where the filing is a completion in the U.S. of an International Application or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.

☒ Application is made by a person authorized under 37 C.F.R. § 1.41(c) on behalf of *all* the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 C.F.R. § 1.16(e) can be filed subsequently).

☐ Showing that the filing is authorized. *(not required unless called into question. 37 C.F.R. § 1.41(d))*

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

☐ The same.

or

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

☐ is submitted.

☐ will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of $130.00 required by 37 C.F.R. § 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 C.F.R. § 1.52(d).

☒ English

☐ Non-English

☐ The attached translation includes a statement that the translation is accurate. 37 C.F.R. § 1.52(d).

8. Assignment

☒ An assignment of the invention to BODENSEEWERK PERKIN-ELMER GmbH

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☒ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: *A newly executed "CERTIFICATE UNDER 37 C.F.R. § 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.*

9. Certified Copy

Certified copy(ies) of application(s)

Country	Appln. No.	Filed
Germany	19903576.8	29 January 1999
Country	Appln. No.	Filed
Country	Appln. No.	Filed

from which priority is claimed

☐ is (are) attached.

☒ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 C.F.R. § 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. § 120 is itself entitled to priority from a prior foreign application, then complete Item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. § 1.16)

A. ☒ Regular application

CLAIMS AS FILED					
Number filed		Number Extra		Rate	Basic Fee 37 C.F.R. § 1.16(a) $ 690.00
Total Claims (37 C.F.R. § 1.16(c))	32 − 20 =	12	×	$ 18.00	216.00
Independent Claims (37 C.F.R. § 1.16(b))	1 − 3 =	0	×	$ 78.00	0
Multiple dependent claim(s), if any (37 C.F.R. § 1.16(d))			+	$260.00	

☐ Amendment cancelling extra claims is enclosed.

☒ Amendment deleting multiple-dependencies is enclosed.

☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 C.F.R. § 1.16(d).

Filing Fee Calculation $ 906.00

B. ☐ Design application
($310.00—37 C.F.R. § 1.16(f))

Filing Fee Calculation $_____

C. ☐ Plant application
($480.00—37 C.F.R. § 1.16(g))

Filing fee calculation $_____

11. Small Entity Statement(s)

☐ Statement(s) that this is a filing by a small entity under 37 C.F.R. § 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. § 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).*

WARNING: *"Small entity status must not be established when the person or persons signing the . . . statement can* ***unequivocally*** *make the required self-certification." M.P.E.P., § 509.03, 6th ed., rev. 2, July 1996 (emphasis added).*

(complete the following, if applicable)

☐ Status as a small entity was claimed in prior application

_____ / _____, filed on _____, from which benefit is being claimed for this application under:

35 U.S.C. § ☐ 119(e),
☐ 120,
☐ 121,
☐ 365(c),

and which status as a small entity is still proper and desired.

☐ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A**, **B** or **C** above)

$_____

NOTE: Any excess of the full fee paid will be refunded if small entitiy status is established and a refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 C.F.R. § 1.28(a).

12. Request for International-Type Search (37 C.F.R. § 1.104(d))

(complete, if applicable)

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

☐ Not Enclosed

☐ No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. § 1.16(e) can be paid subsequently.)

☒ Enclosed

☒ Filing fee $ 906.00

☐ Recording assignment
($40.00; 37 C.F.R. § 1.21(h))
(See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION".) $ _____

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached
($130.00; 37 C.F.R. §§ 1.47 and 1.17(i)) $ _____

☐ For processing an application with a specification in a non-English language
($130.00; 37 C.F.R. §§ 1.52(d) and 1.17(k)) $ _____

☐ Processing and retention fee
($130.00; 37 C.F.R. §§ 1.53(d) and 1.21(l)) $ _____

☐ Fee for international-type search report
($40.00; 37 C.F.R. § 1.21(e)) $ _____

NOTE: 37 C.F.R. § 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 C.F.R. § 1.53(f) and this, as well as the changes to 37 C.F.R. §§ 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total fees enclosed $ 906.00

14. Method of Payment of Fees

☒ Check in the amount of $ 906.00

☐ Charge Account No. _____ in the amount of $_____.
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 C.F.R. § 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should not be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

☒ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 16-1350 :

☒ 37 C.F.R. § 1.16(a), (f) or (g) (filing fees)

☒ 37 C.F.R. § 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

☒ 37 C.F.R. § 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☒ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a)).

☐ 37 C.F.R. § 1.17 (application processing fees)

NOTE: ". . .A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . the issue fee. . . " From the wording of 37 C.F.R. § 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: ". . . Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ Credit Account No. 16-1350

☐ Refund

SEND ALL CORRESPONDENCE TO:

Reg. No. 24,622

Tel. No. (203) 259-1800

Customer No.

SIGNATURE OF PRACTITIONER

Clarence A. Green

(type or print name of attorney)

PERMAN & GREEN, LLP

P.O. Address

425 Post Road, Fairfield, Connecticut 06430

☐ **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☐ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added _____

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added _____

☐ Plus added pages deleting names of inventor(s) named in prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added _____

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added _____

☒ **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

☒ This transmittal ends with this page.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Express Mail No.: EL336861901US

In re Application of: STEMMLER et al.

SERIAL NUMBER: EXAMINER:

FILING DATE: Herewith ART UNIT:

TITLE: QUANTITATIVE DETERMINATION OF ANALYTES IN A HETEROGENEOUS SYSTEM

ATTORNEY DOCKET NO.: 739-009159-US(PAR)

The Commissioner of Patents and Trademarks

Washington, D.C. 20231

PRELIMINARY AMENDMENT

Dear Sir:

Please amend the above-identified, enclosed patent application as follows:

IN THE CLAIMS:

Please amend Claims 3 through 8, 10, 11, 12, 14, 15, 17 through 20, 22, 23, 24, 27 through 30 and 32 as shown below.

Claim 3, line 1, delete "or 2".

Claim 4, line 1, delete "one of Claims 1 through 3" and insert --Claim 1 --.

Claim 5, line 1, delete "one of Claims 1 through 4" and insert --Claim 1--.

Claim 6, line 1, delete "one of the Claims 1 through 5" and insert --Claim 1--.

Claim 7, line 1, delete "one of the Claims 1 through 5" and insert --Claim 1--.

Claim 8, line 1, delete "one of the Claims 1 through 7" and insert ---Claim 1--.

Claim 10, line 1, delete "or 9".

Claim 11, line 1, delete "one of the Claims 1 through 10" and insert --Claim 1--.

Claim 12, line 1, delete "one of the Claims 1 through 11" and insert --Claim 1--.

Claim 14, line 1, delete "or 13".

Claim 15, line 1, delete "or 13".

Claim 17, line 1, delete "one of the Claims 1 through 16" and insert --Claim 1--.

Claim 18, line 1, delete "one of the Claims 11 through 17" and insert --Claim 11".

Claim 19, line 1, delete "one of the Claims 1 through 18" and insert --Claim 1--.

Claim 20, line 1, delete "one of the Claims 9 through 19" and insert --Claim 9--.

Claim 22, line 1, delete "or 21".

Claim 23, line 1, delete "one of the Claims 20 through 22" and insert --Claim 20--.

Claim 24, line 2, delete "one of the Claims 1 through 23" and insert --Claim 1--.

Claim 27, line 1, delete "or 26".

Claim 28, line 1, delete "one of the Claims 24 through 27" and insert --Claim 24--.

Claim 29, line 1, delete "one of the Claims 24 through 28" and insert --Claim 24--.

Claim 30, line 1, delete "one of the Claims 24 through 29" and insert --Claim 24--.

Claim 32, line 1, delete "one of the Claims 24 through 31" and insert --Claim 24--.

Remarks

Please enter this preliminary amendment prior to calculation of the fees.

Respectfully submitted,

27 Jan 00

Clarence A. Green, Reg. No. 24,622
Perman & Green, LLP
425 Post Road
Fairfield, CT 06430
(203) 259-1800

Date

Quantitative determination of analytes in a heterogeneous system

The present invention relates to a method for quantitative determination of an analyte in a system comprising at least two different phases. Additionally, the present invention relates to a test sample carrier that is particularly suited to carrying out the method which constitutes the invention.

The prior art describes numerous methods for quantitative determination of an analyte in a specified analysis sample. The various detection reactions are based on different principles. These include conversion of the analyte to be detected to a demonstrable substance, in which case, for instance, a coloured compound is produced and the degree of colouration is a measurement of the quantity of the analyte in question. Other detection methods are based on specific interactions between the analyte and a bonding partner. These include, for example, the detection method utilising the specific interaction between an antigen and an antibody, a ligand and its associated receptor or the hybridisation of complementary nucleic acid molecules. This type of detection method or assays are generally also described as affinity assays. With the affinity reactions on which they are based, there is generally the production of a stoichiometrically defined but not covalent complex formed from bonding partners specific to the analyte (e.g. a receptor, antibody) and the analyte (e.g. a ligand, antigen). Frequently, biomolecules like proteins take part in these reactions. But reactions can also exist between low-molecular substances, e.g. low-molecular receptor ligands, and a high-molecular substance, e.g. the receptor. A special variation of this affinity assay is based on immuno-assay in which the specific interaction between an antibody and an antigen is exploited. At the level of nucleic acid, a specific interaction can take place between two different nucleic acid molecules by means of mutually

complementary sequential segments. By means of hybridisation of the complementary sequences, the formation of a double-stringed nucleic acid molecule results.

The assays cited are applied in numerous technical fields. These include clinical analysis / diagnostics, environmental analysis, genome analysis, active ingredient testing and even gene expression studies and gene bank screenings. Frequently several hundred samples are tested in parallel on a single sample carrier. This so-called "micro-array technique" nowadays achieves increased significance in so-called "high-throughput screenings".

The advantage with assays based on affinity reactions when compared with assays based on chemical conversion of the analyte is that more elaborate preparation of the sample is generally not required. Separation of the analyte from undesirable impurities is rather accomplished by means of the specific interaction with a suitable bonding partner, the latter deliberately selecting, as it were, the analyte desired from the analysis sample.

Immuno-assays constitute a particularly widespread variant of affinity assays based on the specific interaction of antibodies and antigens. In the case of so-called ELISA (Enzyme Linked Immuno-Sorbent Assay), one of the reactants (i.e. either the analyte or the associate bonding partner) is in the sample carrier, frequently constituting a micro-titre plate, in immobilised form. In the course of the test, one or more components of the test system form a complex with the immobilised component. The quantity of the complex formed serves as a measurement of the concentration of the analyte in the sample. Two common variants of this test format are made up of the "sandwich assay" and the "competitive assay". With the sandwich assay, for instance, the analyte is complexed by two further components (often two different antibodies) so that a ternary complex is generated on the sample carrier's surface. With the competitive assays, the analyte and a labelled

component, frequently an analyte carrying a marker, compete for a limited number of bonding positions.

A standard immuno-assay in heterogeneous phase frequently comprises the following processes:

- Specification of a solid sample carrier;
- Administration of analysis sample and a detection reagent;
- Waiting for the binding equilibrium to set;
- Rinsing out unbonded segments;
- Measuring the bonded segments.

Where applicable, these steps can be repeated several times with complex protocols. At the end of the entire process, detection of the entire material then occurs which has been bound to the sample carrier in the course of the procedure. For this, a colouring enzyme reaction, electrochemical luminescence, fluorescence, radioactivity, etc. can be used as a signalling transmitter.

Some assay formats work in homogeneous phase, such as, for instance, the FPIA fluorescence polarisation immuno-assay, but which are in many respects complex or less flexible in the way of modification than assays in heterogeneous phase.

Common to practically all assays cited is that prior to the measurement signal separation of unbonded label (activity, measurement signal) and bonded label must take place. This is generally achieved by having the sample carrier subjected to one or more washing actions prior to measurement (taking the measurement signal). These washing actions, absolutely required in the current prior art, however, entail disadvantages. With sample carriers allowing for numerous detection reactions in a small space, as happens for instance with micro-titre or nano-titre plates, there exists the problem of "transfer," i.e. sample activity is transferred by washing from one sample volume to another one. A further disadvantage of the processes in which physical separation of unbonded and bonded label (or activity)

occurs, consists of the fact that no time-staggered observation of the bonding process and hence no examination of the interaction or reaction kinetics is possible.

Besides the above cited washing for separation of bonded and unbonded activity, with assays using filter strips, separation takes place between unbonded activity and bonded activity by means of diffusion of the liquid phase in the porous solid phase formed by the filter. Assays of this type are usually set up for single samples.

The present invention is based on the technical problem of indicating a method for quantitative or qualitative determination of an analyte or its reaction or interaction kinetics which no longer shows the disadvantages of the present prior art, in particular one which avoids the washing steps required under the prior art.

A further problem consists in indicating a method of the type cited above by which sample volumes of $< 1\ \mu l$ in micro-array arrangement can be analysed.

A further problem consists also in indicating agents which are suitable for conducting the method constituting the invention.

The technical problem cited above is solved in this invention by a method for quantitative or qualitative determination of an analyte or its interaction or reaction kinetics in a system with at least two different phases, comprising the step of taking at least one measurement signal from at least one of the phases, in which context the different phases when taking the measurement signal are present in parallel and each measurement signal is attributed to one of at least two phases.

The method cited constituting the invention allows for said determinations of an analyte without physical separation between unbonded and bonded label taking place prior to taking at least one such measurement signal.

The method is suitable for qualitative detection of the presence of an analyte in a sample. Furthermore, through temporarily successive taking of measurement signals when a binding equilibrium set in or with the unfolding of chemical reactions, the different process kinetics can be established. However, the method preferably serves for quantitative determination of an analyte in an analysis sample.

A common process scenario can be described as follows: After administration of the sample to be analysed, for instance into the well of a nano-titre plate which, by way of example, is coated with a specific bonding partner for the analyte, in the case of a competitive assay, a defined quantity of the labelled analyte is added. After the binding equilibrium sets in, at least one measurement signal is taken from a volume segment of the liquid phase, in which case the signal generated by the solid phase is essentially not taken. The measurement signal taken from the one phase, in the case of our example: the liquid phase, then serves for calculation of the quantity of analyte contained in that phase and ultimately for determination of the quantities of the analyte present in the sample under study. The quantitative evaluation of the measurement signal obtained occurs with a previously created calibration curve in which, under measurement conditions which remain constant, the strength of the signal of submitted and defined analyte concentrations is calculated.

Even qualitative analysis is possible in this way. If, for instance, an analyte is detected in a so-called sandwich format, then for example, a second labelled antibody can only bond to the first unlabelled antibody in the solid phase if an analyte is present. Detection of a signal in the solid phase thus means that an analyte must be present in the sample.

Kinetics can ultimately be calculated in this way in which, for instance, taking of the measurement signal in the liquid phase can be pursued via a temporal sequence. Modification of the

signal in the liquid phase over time is the measure of the kinetics of a reaction or interaction of the analyte under study.

Measurement of a specific quantitative unit of one of the phases can in this way be ensured through corresponding adjustment of the measurement device. Thus, for instance, a laser beam which can be used to stimulate fluorescent-labelled molecules in the analysis sample, can be so arranged that only a certain quantitative segment of a phase, e.g. the liquid phase, is hit by the laser beam and only the molecules in the trajectory of the beam are excited into fluorescence. The fluorescent signal obtained in this way can then serve for calculation of the analyte concentration present.

In a particularly preferred embodiment, the method is carried out as an affinity assay. In doing so, the specific interaction between analyte and one bonding partner is exploited. Examples for an analyte and an associated bonding partner are a ligand as an analyte and a receptor as an associated bonding partner, nucleic acid as an analyte and complementary nucleic acid as a bonding partner, substratum as analyte and associated enzyme as a bonding partner, antigen as analyte and antibody as the corresponding bonding partner. A detailed survey of common processes of affinity analysis is found in C P Price & D J Newman (editors), "Principles and Practice of Immunoassay", Stockton Press, New York, 1991.

In an additional preferred embodiment, the method is carried out as an immuno-affinity assay. In this kind of assay, for detection of the analyte the specific interaction between antigen and antibody is exploited. As antibody for the specific detection of the analyte, so-called monoclonal as well as polyclonal antibodies can be used. Even the analyte serving as antigen can in turn constitute an antibody.

In a further preferred embodiment, the volume in which the detection reaction occurs, amounts to 1 µl or less, the volume

preferably being some 50-100 nl. The volume of the detection reaction corresponds to the sample volume deployed including the reagents added. The prior art knows no process in which such small sample volumes can be subjected to one of the determinations cited above, particularly not if the samples are in a micro-array as when in a nano-titre plate.

With a further preferred embodiment, the method is carried out as a competitive assay. In doing so, the analyte to be detected competes with a structurally similar compound which normally carries a label, for a limited number of bonding positions. Since the added and labelled substance competing with the analyte is administered in constant quantities, the number of bonding positions occupied by the analyte depends on the number of bonding positions taken up by the analyte in the analyte concentration. This means that the more analyte that is present in the sample, the fewer the bonding positions which are occupied by the labelled competitive substance. This in turn means, that with increasing analyte concentration, the quantity of unbonded, labelled competitor in the liquid phase increases. This results in the measurement signal from the liquid phase increasing with an increase in the volume of analyte in the sample. Competitive assays are long since known to the person skilled in the art from the prior art.

However, the method can alternatively be carried out as a sandwich assay. In doing so, for instance, the analyte to be determined is implemented with two different antibodies. Here, a first antibody is bound to the solid phase, and the second antibody bears a label and is administered to the analysis sample. When interaction is completed, a ternary complex is formed in the solid phase consisting of the first antibody, the analyte and the labelled second antibody. In doing so, the signal in the solid phase increases with increasing analyte concentration while the concentration of the labelled second antibody in the liquid phase decreases with increasing analyte concentration. If a volume element of the liquid phase is measured in detection, then decreasing signal strength is

observed with increasing analyte concentration. The sandwich assay principle is well known to the person skilled in the art from the prior art.

The measurement signal is preferably obtained by a label which is present as a component of the analyte or of the bonding partner (reactant). Suitable labelling possibilities are known to the person skilled in the art from the prior art. They include radioactive label, label produced by irradiation excitement such as labelling done, for instance, by fluorescent markers, or labelling with an enzyme activity. As a particularly preferred label, a fluorescent group is introduced into the molecule in question. Here fluorescence can be generated for example by having the labelled molecules excited by a laser beam. This technique is also well known to the person skilled in the art from the prior art.

In a further preferred embodiment, the system with which the process is carried out comprises a first phase constituting a solid phase and a second phase constituting a liquid phase. Here the solid phase generally bears the specific bonding partner for the analyte to be detected while the liquid phase is formed by the sample containing the analyte and the detection reagents. In a further alternative, the second phase can, however, also be a gaseous phase while the first phase constitutes the solid phase. Finally, the combination of phases can also consist of the combination of a liquid phase with a gaseous phase.

In a further preferred embodiment, the solid phase is formed by the walling of a well in a solid sample carrier. In doing so, the solid sample carrier can be fashioned so that it is only suited to absorbing a single sample. But the sample carrier can also be formed so that several samples can be absorbed simultaneously.

In a particularly preferred embodiment, the solid sample carrier is a micro-titre plate of the type commercially available. Particularly preferred is a nano-titre plate being used as a

sample carrier, since the former has a large number of wells for absorbing the sample in a small space. The wells in the solid sample carrier can have different shapes. These include the quadratic or cylindric shape, truncated pyramid or truncated cone. Particularly preferred are, additionally, shapes whose aperture surface is smaller than their bottom surface; these include by way of example the negative truncated pyramid and the negative truncated cone. With this embodiment, mitigation of the measurement results from stray light / fluorescence on the part of label bound to the solid carrier is minimised by comparison with the corresponding embodiments whose bottom surface is smaller than their opening surface (such as with a positive truncated pyramid or positive truncated cone).

In a further preferred embodiment, the influence of interference effects from the label bound to the phase is lowered by having the phase contain a quenching substance. This quenching substance absorbs the signal generated by the molecules arranged in immediate proximity to the quenching substance. Preferably, the quenching substance is selected so that it quenches the fluorescence obtained when the molecules present in the system are excited by a laser. Preferably, the material quenches the fluorescence within a short distance, preferably less than 100 nm. As preferred materials, metals such as gold or silver, as well as graphite or dyes with "quenching properties" can be considered.

By way of example, the solid phase can contain such a quenching substance. For this purpose, the sample carrier can be coated with quenching material. This is particularly advantageous in cases where only the fluorescence of molecules located in the solution is to be recorded.

Receiving the measurement signal from only one of the signal generating phases present can, for instance, be obtained by space-staggered measurement. This can be done by having a laser beam sense the entire well in which the sample is located and, depending on its resolution capacity, several measurement

signals can be obtained. The individual measurement signals represent the intensity of fluorescence occurring at each position and can thus be used for determination of the local concentration of an analyte.

Basically, however, it suffices if only a single measurement signal corresponding, for instance, to a defined volume element of the liquid phase, is taken.

In addition to this, by taking several signals of a phase, for instance by sensing the sample with a laser beam, the statistics can be improved by averaging out such measurement signals and thus the determination of an analyte or the interaction or reaction kinetics can be improved as well as errors in determining an analyte or interaction or reaction kinetics can be reduced.

In accordance with a further preferred embodiment, the quenching substance can be provided so that radiation of one phase is almost completely suppressed. According to this embodiment, it is not necessary to carry out spatially staggered taking of at least one measurement signal for attribution to the corresponding phase. If, for example, the walling and/or the floor of the well of a sample carrier is coated with quenching material, then the fluorescence resulting from the label bound to the walling can be suppressed and the fluorescence stemming from label in the liquid phase can be taken without requiring staggering of space. In one preferred embodiment, a spot with a diameter of 40 µm or less, preferably of about 20 µm, is illuminated and the generated signal of this volume segment is measured only.

Illuminating the sample volume segment is preferably done with a laser where the generated signal is a fluorescence which is emitted by the fluorophore-bearing molecules excited by the laser.

According to the invention, a sample carrier is also made available having one or more wells and which is characterised by the fact that at least a part of the sample carrier is at least in the range of one or more wells coated with fluorescence-quenching material.

This sample carrier can in particular be used in the processes described above for suppressing the fluorescent radiation of one phase. Of course, the applications of such a sample carrier are not limited to the processes described above; rather, such a sample carrier can also be used in other processes in which, for example, reducing stray radiation requires that fluorescence be quenched in a specific range.

According to an advantageous embodiment, the fluorescence-quenching material can comprise a metal such as, by way of example, gold or silver. If needed, this metal can also be doped.

The well or wells can advantageously be coated with the fluorescence-quenching material in accordance with the requirements in the floor area and/or the walling.

In addition, the advantages already described in connection with the processes can be achieved by different shapes of wells. Thus wells can be provided for in the sample carriers which have a quadratic, cylindrical, truncated pyramid or truncated cone shape. As likewise described above, it is particularly advantageous to provide a well whose aperture surface is smaller than its bottom surface. These include, in particular, a negative truncated pyramid shape or a negative truncated cone shape.

According to a further advantageous embodiment, the sample carrier is shaped in the form of a micro-titre plate, preferably a nano-titre plate. In this way, it becomes possible to analyse a number of samples with an optimum expenditure of time.

Here below, preferred embodiments of the invention and examples of the method constituting the invention are described with reference to the enclosed drawing. The following is shown in the drawing:

Fig 1A schematic view of an arrangement for carrying out the method constituting the invention.

Fig 2A well of a sample carrier according to an embodiment of the present invention.

Fig 3Various shapes of wells of a sample carrier according to different embodiments of the present invention.

Fig 4A calibration graph showing fluorescence intensity as a function of the concentration of the analyte (atrazine) when using a monoclonal antibody according to a first example of the method constituting the invention.

Fig 5A calibration graph showing fluorescence intensity as a function of the concentration of the analyte by using a polyclonal antibody according to a second example of the method constituting the invention.

Fig 6A view for explaining the connection between the concentration of the analyte and the intensity of fluorescence.

Fig 7A first view for explaining the determination of a spatial fluorescence distribution according to a further embodiment of the method constituting the invention.

Fig 8A second depiction for explaining the determination of a spatial fluorescence distribution according to a further embodiment of the method constituting the invention.

1. Components for carrying out the process

a) Sample carrier

For example, a planar sample carrier having one or more wells, which can absorb a liquid volume of 1µl or less, can be used. Its surface is covered with material which massively reduces (quenches) fluorescence at a short distance (below 100 nm). The quenching material is preferably a metal, gold and/or silver; however, it can also be graphite or a dye with quenching properties. The sample carrier's surface is further covered with a bonding partner for the analyte. The bonding partner can be an antigen or an antibody. The bonding partner can either be fixed through adsorption or through covalent bonding to the sample carrier. Suitable coating procedures are known to the person skilled in the art.

b) Reagents

An essential component is constituted by a fluorescence-labelled bonding partner specific to an analyte (e.g. an antibody), which can specifically bond to the analyte. Basically, the bonding partner is in a position to bond to free analytes in the sample as well as to an immobilised analyte on the sample carrier. Processes for manufacturing fluorescence-labelled antibodies are known to the person skilled in the art.

As further reagents, substances can be used which can prevent or reduce non-specific bonding of solid components, i.e. analyte or bonding partners, to the sample carrier. For this purpose, for instance, proteins or detergents can be considered. Substances suitable for suppressing non-specific bonds to the sample carrier are known to the person skilled in the art.

c) Selection device

A lense device is used allowing stimulation of the fluorescence-labelled molecules present in the wells and allowing for detection of the fluorescent light emitted. Here the fluorescence can be excited and/or detected spatially-staggered inside any well. In this way, selective measurement of the fluorophores present in the solution is possible without separation of bound and free fluorophores being necessary. Selective measurement can be done by means of various variations of the measurement device. It is possible to so condition the stimulation lense so that only fluorophores in solution are excited while fluorophores in solid phase are not excited. Alternatively, it is possible by varying the detection lense to only record such fluorescence as is emitted by molecules in solution while fluorescence emitted by solid-phase bound molecules is not recorded. Finally, it is possible by varying the sample carrier accordingly to only record fluorescence stemming basically from molecules found in solution. This is made possible by wells for absorbing the sample which show a negative truncated pyramid in cross-section. Naturally, a combination of the alternatives cited can also achieve the desired effect.

2. Quantitative determination with the aid of a competitive assay

For quantitative determination of a dissolved analyte in a liquid sample, the following steps are carried out:

- A sample carrier is prepared in which one or more wells are coated with a test system component, e.g. an antibody. This coating can be separated temporally and spatially from the carrying out of the actual quantitative determination. Additionally, the sample carrier can be modified in different ways in different wells.

- The sample and the reagents required are introduced into one or more wells. Where needed, the well can then be sealed with a suitable agent for further storage.

- The sample is incubated with the reagents until the binding equilibrium between analyte and bonding partner has set in.

- The fluorescence of the molecules present in the sample is excited or detected under conditions under which either essentially the molecules in solution are excited or the molecules bound to the surface are excited. Possible is also sequential stimulation of the fluorescence molecules distributed among both phases, perhaps by grid-shaped sensing of the sample carrier.

- In the competitive assay described, the fluorescence signal in the sample solution rises with the analyte concentration while the fluorescence signal on the walling of the sample carrier decreases with increasing analyte concentration. Decreasing of the fluorescence signal with increasing analyte concentrations is reinforced if the surface of the sample carrier is coated with a fluorescence quenching substance. The signal obtained can in the usual manner be calibrated with reference measurements and correlated with the concentration of the analyte.

3. Measuring device for carrying out the process

Fig 1 shows schematically a possible measurement arrangement. Such a measurement arrangement is known, for instance, from Dixon's US Patent 5,381,224. This measurement arrangement consists of a laser 10, a mass-produced available beam expander 11, a sensing device or scanner 12, a specially designed test table 13 for securing a sample carrier 14, an imaging lense 15 as well as a detection device for detecting fluorescent radiation 16, and, for example, one or more photo-multipliers. In addition, the system can have a suitable filter combination

17. Fluorescence stimulation by means of the laser 10 occurs in this case via the beam expander 11, the scanner 13 and a beam splitter 18, for example in the form of a dichroitic mirror, on the sample. The fluorescence radiation 19a emitted by the sample is, in the reflected direction, captured by the beam splitter 18 with an imaging lense 15 recessed into the detection device 16. From the position of the laser beam 19b, known from controlled regulation of the scanner 13, every light signal can be attributed unambiguously to a point on the sample carrier 14 and thus to a sample to be measured or to sample volumes. The intensity of the signal obtained in the detection device 16 serves as a quantitative measure for analyte concentration in the sample.

4. Configuration of the sample carrier

The sample carrier used possesses at least one well but will generally comprise more than one well. Here both micro-titre as well as nano-titre plates can be used as sample carriers.

In Fig 2, a partial view of a micro-titre plate 20 is depicted which has a truncated pyramid shaped well 21. The micro-titre plate 20 is covered with a fluorescence-quenching coating 22, for example of gold and/or silver. The coating of the sample carriers with gold as a quenching substance can be carried out with a vacuum metallising unit (Edwards 305) by means of thermal vaporisation of the gold. The sample carriers are first cleaned with laboratory cleaners (Extran, Merk), dried and then introduced into the metallising chamber. At a vacuum of better than 10^{-6} bar, coating thicknesses of 500 nm to 1000 nm of gold have been shown to be useful.

According to Fig 2, the entire surface of the micro-titre plate is coated. This entails simplification in manufacturing the coating. Complete coating of the micro-titre plate is, however, not required. Rather, depending on the specific conditions emerging from a particular analysis, only the floor 23 and/or the walling 24 of the well can also be coated. The sample

carrier shown in Fig 2 is produced by having a silicium substratum 28 pickled by means of the shape of the well by means of different wet-pickling techniques such as anisotropic wet-pickling and the silicium substratum is subsequently provided with a floor 29.

In Fig 2, there are further fluorophores 25 bound to the walling and the floor and fluorophores 26 in solution are depicted.

Of major importance for the quality of the measurement signal obtained is the fashioning of the well. Due to the spatially restricted stimulation with a focussed laser light source, primarily molecules in solution are detected. For reducing the fluorescence stimulation of the molecules bound to the solid phase, vertical walling 32 can be used in the wells 31 as they are shown in the left illustration of Fig 3. Such vertical walling is found in a quadratically or cylindrically shaped well.

For almost complete elimination of fluorescence stimulation of the molecules bound to the walling, special well shapes are suitable whose aperture surface 36 is smaller than their floor surface 37. In the right illustration of Fig 3, for instance, a cross-section of such a well 35 is depicted. This well 35 has a truncated pyramid or truncated cone shape. These varying shapes result in no direct fluorescence stimulation of the molecules bound to the walling taking place and furthermore with no detection of residual fluorescence, initiated by stray light in the aqueous phase, occurring. Consequently, with wells of this shape, fluorescence is practically exclusively measured on molecules in the aqueous phase.

5. Quantitative determination of a pesticide

In the experiment, a pesticide derivative from the category of substituted S-triazines was determined. As bonding partner for such an analyte, antibodies were used.

Polyclonal sheep antibodies were enriched from serum by means of fractionated ammonium sulphate precipitation and isolated via an affinity column (Sephadex column, containing the immunogen).

Monoclonal antibodies were isolated from hybridom culture residues (serum-free culture) and cleaned via a protein G column.

For fluorescence labelling, a commercially available reactive fluorescent dye (CY5-N-hydroxy-succinimide, Amersham-Pharmacia-Biotech) was used. Labelling of the antibodies was done according to the manufacturer's instructions. The labelled antibodies were cleaned by means of spin dialysis (Amicon Concentrators, Aminco). The labelling degree is determined spectro-photometrically.

Conjugates of beef serum albumin (BSA) and haptene were produced as follows: From a triazine carboxyl derivative (atrazine caproic acid), an active ester was produced in DMF with di-isopropyl carbodimide (Sigma) and N-hydroxy-succinimide (Sigma). 1 mg of BSA in 100 mM of carbonate buffer, pH 9.0 was compounded with an excess of active esters and incubated for one hour at room temperature. The conjugate was cleaned by means of spin dialysis (Amicon Concentrator, Aminco). The labelling degrees were determined spectro-photometrically.

Coating of the sample carriers with gold as quenching substance was done in a vacuum metallising unit (Edwards 305) by means of thermal vaporisation of gold. The sample carriers were cleaned with laboratory cleaner (Extran, Merk), dried and introduced into the metallising chamber. At a vacuum of better than 10^{-6}, 500 nm to 1000 nm of gold were metallised. For comparative purposes, in each case a portion of the sample carrier was covered over during the metallisation process. After metallisation, the gold coatings were pickled in ethanol for one day in a solution of 0.2% ˇ-mercaptopropionic acid, washed with ethanol and dried.

The surfaces of the sample carriers, e.g. the micro-titre plates (Greiner Labortechnik) or the nano-titre plates (GeSIM, volume of the cup 600 µm x 600 µm at a depth of 400 µm, equivalent to about 50 nl volume, truncated pyramid shaped wells, anisotropically pickled in silicium), were covered with the conjugate as follows: The surface was incubated for one hour with a solution of the conjugate in phosphate-buffered brine, pH 7.4. Subsequently, the surface was washed and incubated for an additional hour with a solution of 1 mg/ml BSA in order to saturate off non-specific bonding positions. For stabilising the coating, for one hour it was incubated with a solution of 0.5% glutaranhydride (Sigma). Subsequently, the sample carriers were washed and either immediately used or dried and stored at 4°C. For comparative experiments, the surfaces were only coated with BSA but not with a BSA pesticide conjugate.

5.1 Quantitative determination of an atrazine derivative as haptene with mechanical separation of bound and free antibodies

In the experiment, the atrazine coated micro-titre plates described above were used. The experiment describes the quantitative determination of antibodies directed at atrazine, in which case the antibodies in the dissolved fraction were determined after separation from the solid phase.

Into each well of a micro-titre plate with an atrazine derivative, as described above, at first 100 µl of an atrazine solution was introduced, followed by 100 µl of a solution of fluorescence-labelled anti-atrazine antibodies. The final concentration of atrazine in the solution varied between 0.003 µg/l and 1000 µg/l. The antibody concentration amounted in each case to 500 ng/ml. After an incubation period of one hour at room temperature, from each well of the micro-titre plate 150 µl of solution was removed and introduced into the well of an opaque white fluorescence micro-titre plate (Perkin Elmer). Fluorescence was measured with a micro-titre plate fluorescence

photometer (Perkin Elmer LSR 2000, stimulation at 670 nm, detection at 700 nm). Fig 4 shows the calibration graph obtained for one monoclonal antibody; Fig 5 shows the calibration graph for one polyclonal antibody. Fluorescence intensities are specified in arbitrary units. Both calibration graphs show a clear and significant link between intensity of fluorescence and the concentration of the analyte (atrazine).

Both in the case of using monoclonal antibodies as well as when using polyclonal antibodies, quantitative determination of the atrazine was possible in a concentration of less than 1 µg/l.

5.2 Determination of the spatial fluorescence distribution on a miniaturised sample carrier

In this experiment, it is shown that settling of fluorescence-labelled bond molecules on the walling of the sample carrier entails spatial distribution of the fluorescent signal, such distribution deviating in a clear and measurable manner from spatial distribution without the settling of fluorescence-labelled bond molecules to the walling.

A GeSIM sample carrier was, as described above, treated and covered on one half with BSA only and on the other half with BSA atrazine conjugate. Into each well was introduced with a piezo-microdrop system (MICRODROP) 50 nl of a solution of fluorescence-labelled anti-atrazine antibodies in phosphate buffered brine (pH 7.4) with 100 µg/ml ovalbumin. The antibody concentrations were 0.2 µg/ml 0.5 1 µg/ml and 1.0 µg/ml. Subsequently, the plate was sealed with a transparent adhesive tape (Adhesive Research) and incubated for 30 minutes at room temperature. Subsequently, a spatial image of the intensity of fluorescence of the plate was produced with a laser scanner by sensing line-for-line the sample carrier (stimulation by means of the adhesive tape at 632 nm, detection with a photo-multiplier at 690 nm). In the structure used, a spot with a diameter of about 20 µm was illuminated by laser on the sample carrier. By means of a computer-assisted data recording system,

an image of the fluorescence intensity distribution was sketched with spatial resolution of 50 µm in arbitrary units. For each well, an image of about 10 x 10 pixels resulted from this.

For evaluation, the intensity of the fluorescence for each square well was determined by summation across all 100 attributed pixels. The result of this summation is depicted in Fig 6. In Fig 6, "atrazine" refers to the signals from the wells coated with an atrazine protein conjugate. "OVA" refers to the wells coated exclusively with ovalbumin. The total fluorescence for both, i.e. wells coated with atrazine protein conjugate as well as for those exclusively coated with ovalbumin, increases with antibody concentration.

In order to determine the spatial distribution of fluorescence, the mean intensity (I_4) per pixel for a square of 4 x 4 pixels in the middle of each well was determined, the mean intensity (I_{10}) being determined for the entire well (10 x 10 pixels). From these two readings, the quotient I_4/I_{10} was determined. These results are depicted in Fig 7.

In Fig 7, "atrazine" again refers to the signals from the wells coated with an atrazine protein conjugate and "OVA" to wells coated exclusively with ovalbumin. For wells in which no bonding of the antibody to the walling occurs (OVA; see left illustration in Fig 8), the mean fluorescence in comparison with the wells in which the antibody bonds to the walling (atrazine; see right illustration in Fig 8) is increased, This effect is largely independent of the concentration.

To be noted in this context is that the laser beam is widened by the concave surface of the liquid meniscus upon entering the liquid sample. By means of this and by means of reflection in the well, the local selectivity of stimulation is limited. By means of the variation of the sample carrier indicated as constituting the invention, as well as of the stimulation and/or detection system, a significantly better signal profile can be achieved. For all concentrations of antibodies used, as

expected, a decline in fluorescence asymmetry, i.e. a lesser emphasis of the mean of deeper bonding of the antibodies to the walling took place.

5.3 Quenching of the fluorescence signal with settling of fluorescence-labelled bonding molecules to walling of a miniaturised sample carrier coated with fluorescence-quenching material

The experiment serves to show that the settling of fluorescence-labelled bonding molecules to gold coated walling of the sample carrier entails significant decrease in the fluorescence signal, which decrease can be prevented by administering an analyte in liquid phase, something which makes the method suitable for quantitative determinations.

A GeSIM sample carrier was vaporised with gold, as described above, further treated and covered on one half with BSA only, and on the other half with BSA atrazine conjugate. Into each well was introduced with a piezo-microdrop system (MICRODROP) 50 nl of a solution of fluorescence-labelled anti-atrazine antibodies (1 µg/ml) in phosphate buffered brine (pH 7.4) with 100 µg/ml of ovalbumin. Into a portion of the wells there was additionally introduced an atrazine derivative with a concentration of 100 ng/ml. The following table provides the average readings for each of the 10 wells.

	Fluorescence (arbitrary units)	% error
1 mg/ml ovalbumin/PBS (background)	5149	1.7
1 µg/ml fluorescence-labelled antibody without atrazine derivative	43574	1.8

1 µg/ml fluorescence-labelled antibody
with 100 ng/ml of atrazine derivative 65866

1.2

The fluorescence of the antibody decreases upon bonding to the walling by about 33% ("without atrazine derivative"). The correspondingly higher fluorescence upon adding of the atrazine derivative is explained by the blocking of the antibody bonding positions. In this way, the antibodies are not bound closer to the fluorescence-quenching walling of the sample carrier. When a sample carrier which has only been coated with BSA is used, no comparable effect was found. The reproducibility of the method is satisfactory and allows for quantification of modifications of fluorescence.

Patent Claims

1. Method for quantitative or qualitative determination of an analyte or its interaction or reaction kinetics in a system with at least two different phases, comprising the step of taking at least one measurement signal from at least one of the phases, in which case the different phases are present in parallel when the measurement signal is taken and each measurement signal is attributed to one of the at least two phases.

2. Method according to Claim 1 in which the method is conducted as an affinity assay.

3. Method according to Claim 1 or 2 in which the analyte constitutes a nucleic acid.

4. Method according to one of Claims 1 through 3 in which the method is conducted as an immuno-affinity assay.

5. Method according to one of Claims 1 through 4 in which the volume in which the detection reaction occurs is less than 1 µl, preferably in the range of 50 to 100 nl.

6. Method according to one of the Claims 1 through 5 in which the method is conducted as a competitive assay.

7. Method according to one of the Claims 1 through 5 in which the method is conducted as a sandwich assay.

8. Method according to one of the Claims 1 through 7 in which the analyte or the reactant carries a label by which the measurement signal is generated.

9. Method according to Claim 8 in which the measurement signal is generated by irradiation excitement of the label.

10. Method according to Claim 8 or 9 in which, as label, a fluorescent label is provided.

11. Method according to one of the Claims 1 through 10 in which a first phase is provided as a solid phase and a second phase as a liquid phase.

12. Method according to one of the Claims 1 through 11 in which the solid phase is formed by walling of a well in a sample carrier.

13. Method according to Claim 12 in which the sample carrier is provided in the form of a micro-titre plate, preferably a nano-titre plate.

14. Method according to Claim 12 or 13 in which a well is provided which has a quadratic, cylindrical, truncated pyramid or truncated cone shape.

15. Method according to Claim 12 or 13 in which a well is provided whose aperture surface is smaller than its floor surface.

16. Method according to Claim 15 in which a well is provided having a truncated pyramid or truncated cone shape.

17. Method according to one of the Claims 1 through 16 in which a quenching substance is linked to a phase for suppressing measurement signals of one of the at least two phases.

18. Method according to one of the Claims 11 through 17 in which a well is provided whose walling and/or floor is coated with a quenching substance, preferably a fluorescence-quenching substance.

19. Method according to one of the Claims 1 through 18 in which at least one measurement signal is obtained by spatially staggered measurement.

20. Method according to one of the Claims 9 through 19 in which the sample quantity containing the labelled analyte or the labelled reactant is radiated with a light beam for stimulation of the label and the reacting radiation of the labelling is taken as a measurement signal.

21. Method according to Claim 20 in which the stimulating light beam in the sample volume has a beam diameter of < 40 µm, preferably of about 20 µm.

22. Method according to Claim 20 or 21 in which the exciting light beam for taking a plurality of measurement signals is conducted via the sample.

23. Method according to one of the Claims 20 through 22 in which stimulation occurs with a laser and as a measurement signal fluorescence of the label excited by the laser beam is taken.

24. Sample carrier (20), in particular for use in a method according to one of the Claims 1 through 23 with one or more wells (21)

characterised by the fact that

at least a portion of the sample carrier (20) at least in the range of one or more wells (21) is coated with fluorescence-quenching material.

25. Sample carrier according to Claim 24 in which the fluorescence-quenching material comprises a metal.

26. Sample carrier according to Claim 25 in which the metal is doped.

27. Sample carrier according to Claim 25 or 26 in which the metal comprises gold and/or silver.

28. Sample carrier according to one of the Claims 24 through 27 in which the range comprises the floor (23) and/or the walling (24) of one or more wells (21).

29. Sample carrier according to one of the Claims 24 through 28 in which one or more wells have a quadratic (31), cylindrical (31), truncated pyramid (21, 35) or truncated cone (21, 35) shape.

30. Sample carrier according to one of the Claims 24 through 29 in which the well has an aperture surface (36) which is smaller than the floor surface (37) of the well.

31. Sample carrier according to Claim 30 in which the well has a truncated pyramid (35) or truncated cone (35) shape.

32. Sample carrier according to one of the Claims 24 through 31 in which the sample carrier is designed in the form of a micro-titre plate, preferably a nano-titre plate.

Abstract

The invention concerns a method for quantitative or qualitative determination of an analyte or its interaction or reaction kinetics in a system with at least two different phases, comprising the step of taking at least one measurement signal from at least one of the phases, whereby the different phases are present in parallel when taking the signal and whereby each measurement signal is attributed to one of at least two phases. In addition, the invention concerns a sample carrier, in particular for use in the method constituting the invention with one or more wells. The sample carrier is characterised by the fact that at least a portion of the sample carrier at least in the range of one or more wells is coated with fluorescence-quenching material.

Figure 4 Arbitrary Units
Intensity of Fluorescence
Atrazine , ...

Figure 5 Intensity of Fluorescence (arbitrary units)

Figure 6 Total Fluorescence
Atrazine
Antibodies

Figure 7 Distribution of Fluorescence
Atrazine
Antibodies

Figure 8 Distribution of Intensity
Distribution of Fluorophores

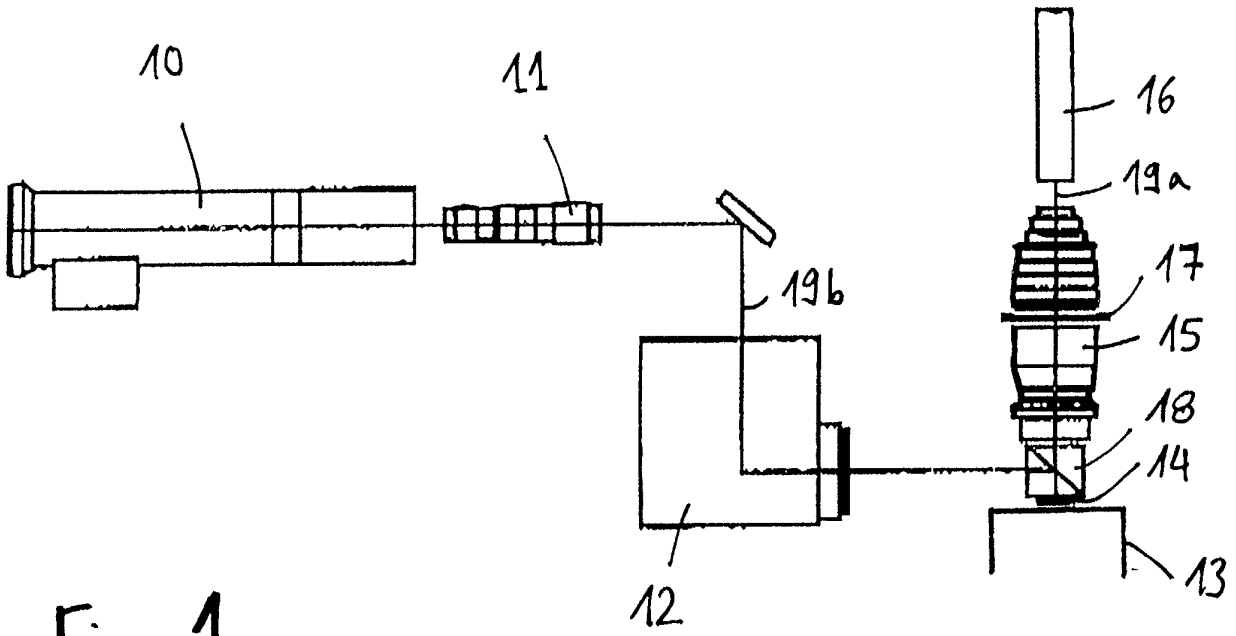


Fig. 1

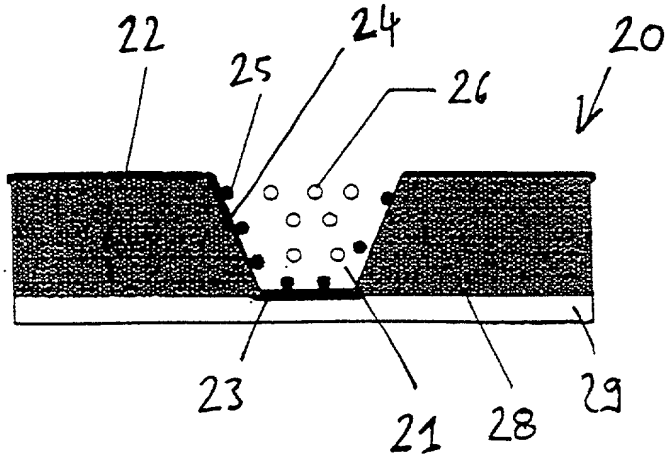
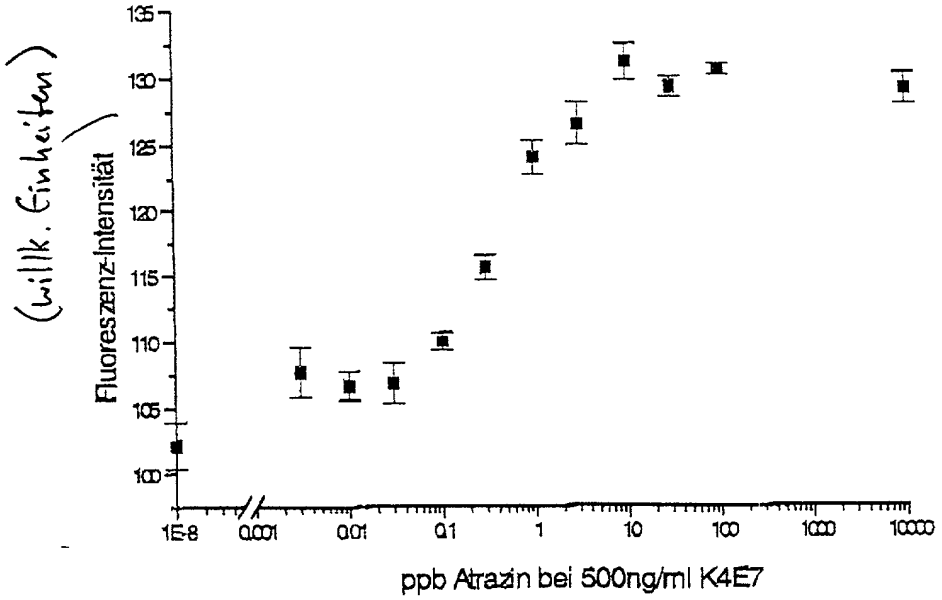


Fig. 2

Fig. 3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125
120
115
110
105
100
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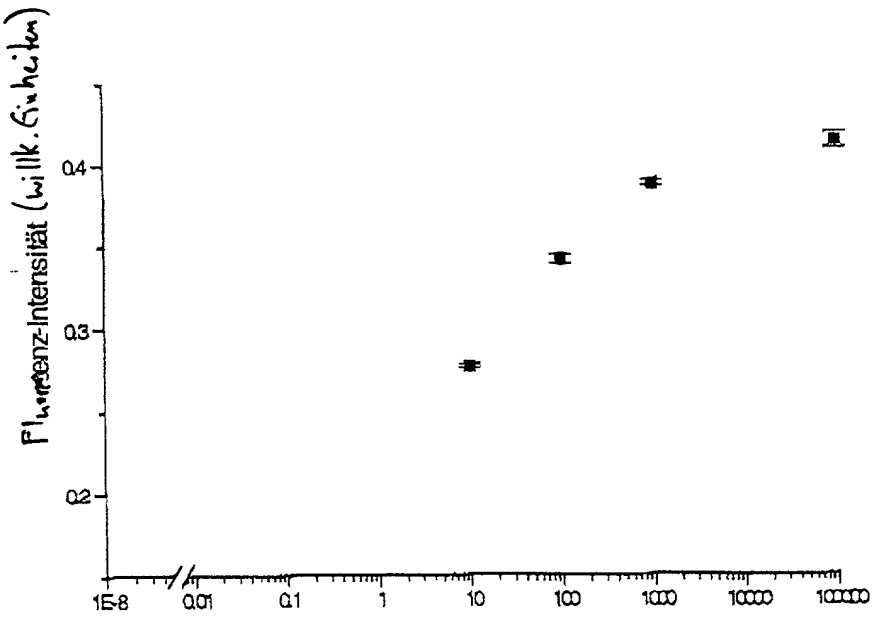
0.01
0.1
1
10
100
1000
10000
ppb Atrazin bei 500ng/ml K4E7

Fig. 4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0.2
1E-8
0.01
0.1
1
10
100
1000
10000
100000

Fig. 5

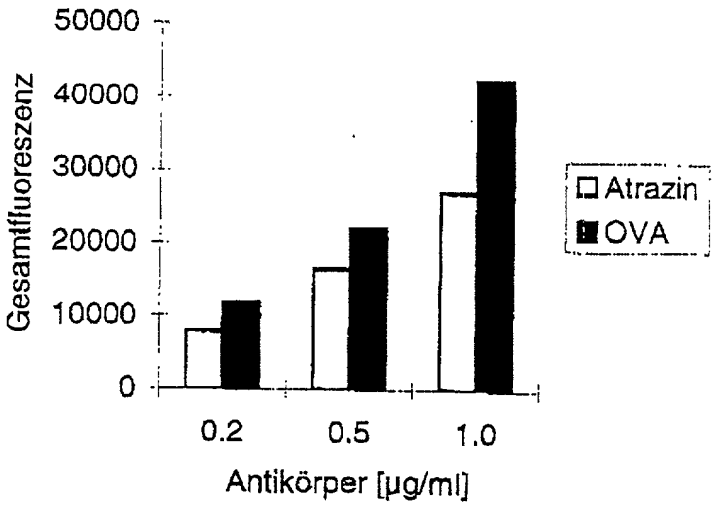
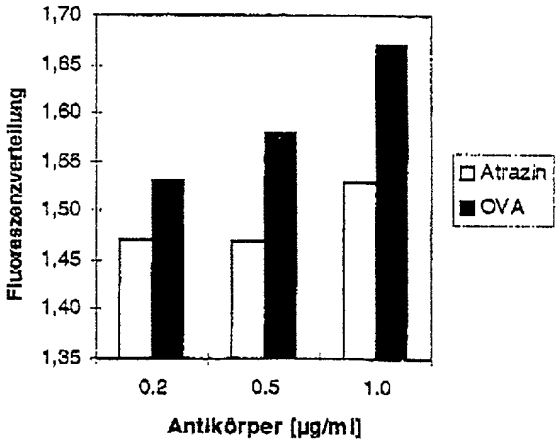

50000
40000
30000
20000
10000
0
Gesamtfluoreszenz
0.2
0.5
1.0
Antikörper [µg/ml]
Atrazin
OVA

Fig. 6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1,60
1,55
1,50
1,45
1,40
1,35
Fluoreszenzverteilung
0.2
0.5
1.0
Antikörper [µg/ml]
Atrazin
OVA

Fig. 7

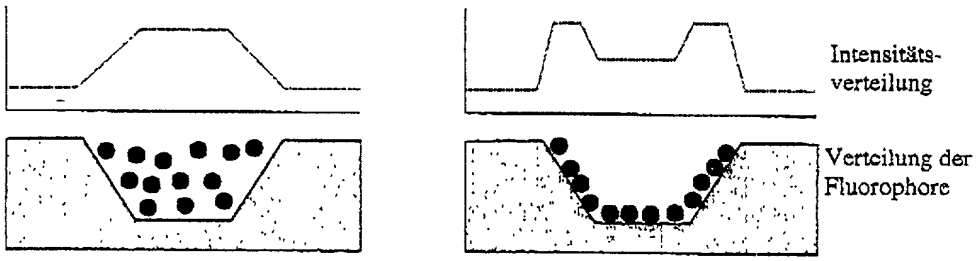
Intensitäts-
verteilung
Verteilung der
Fluorophore

Fig. 8